IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Hauptmann *et al.*	Art Unit: 1812
Appl. No.: 08/249,671	Examiner: Fitzgerald, D.
Filed: May 26, 1994	Atty Docket: 0652.1350000/RWE/LLK
For: **Process for Preparing and Purifying Alpha-Interferon**	

Declaration Under 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

The undersigned, Rudolf Hauptmann, declares and states that:

1. I received a Dr.rer.nat. (equivalent to a Ph.D.) at the University of Vienna, located in Vienna, Austria, in 1976.

2. I am a coinventor of the above-captioned patent application.

3. Since 1982, I have been employed by the Ernst Boehringer Institut für Arzneimittelforschung of Bender & Co. GmbH in Vienna, Austria. A copy of my curriculum vitae is attached hereto as Exhibit A.

4. I have read and I am familiar with the prosecution of this application, including the Office Action of May 16, 1996, wherein the Examiner rejected claims 1-3 and 17-19 as obvious over

Miyake *et al.* in view of Chang *et al.*, and further in view of Vandlen *et al.*, Capon *et al.*, and Baxter *et al.*

5. Table 1 of Chang *et al.* shows that the amount of human growth hormone (hGH) produced using a vector comprising a human hGH cDNA ligated to a sequence encoding the STII signal sequence (STII/hGH), under the control of a trp promoter, is twice as high (1 gram/50 OD/l) as the amount of hGH produced using a vector comprising STII/hGH under the control of a phoA promoter (0.5 gram/OD/l).

6. In contrast, we have found that a 3 fold higher level of IFNα expression is achieved by using the claimed vector construct comprising IFNα cDNA ligated to a sequence encoding the STII signal sequence (STII/IFNα) under the control of a phoA promoter as compared to a STII/IFNα construct under the control of a trp promoter (see Exhibit B, attached hereto, which details the construction of the relevant plasmids, the experiments performed, including controls, and the results obtained). Therefore, while Chang *et al.* would have suggested to one of ordinary skill in the art that the highest level of expression of a mammalian protein could be obtained by linking the gene of interest to a STII leader sequence and expressing this construct from a trp promoter, the present inventors have unexpectedly discovered that much better expression levels of IFNα may be obtained by expressing a STII/IFNα from a phoA promoter.

7. Therefore, it would not have been obvious to one of ordinary skill in the art at the time the invention was made to construct an expression vector for IFNα according to Miyake *et al.*, replacing the phoA signal-peptide encoding sequence employed by Miyake *et al.* with the STII signal sequence used by Chang *et al.* Rather, if one of ordinary skill in the art were to assume that

the findings of Chang *et al.* regarding hGH expression would be relevant to the expression of IFNα, the logical construct to make would have been a STII/IFNα fusion under the control of a trp promoter.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

_____ _____

Date Rudolf Hauptmann

135.DEC

Exhibit A

CURRICULUM VITAE of Rudolf Hauptmann

1950	Born March 17 at Vienna / Austria.
1968	"Matura mit Auszeichnung (cum laudae) at the Bundesrealschule Wien 3., Radetzkystraße 2.
1968-1973	Study of Chemistry at the University of Vienna.
1973-1976	Thesis at the Institute of Biochemistry.
1976	Dr.rer.nat. (Ph.D.) at the University of Vienna.
1976-1977	"Assistent" at the Institute of Biochemistry.
1977-1980	"Assistent" at the Institute of Molecular Biology at the University of Vienna.
1980-1982	Post doctoral study at the University of Leicester, Leicester, UK, about cloning and sequencing of Influenza and Polio viruses.
Since 1982	Leader of the Molecular Biology laboratory I at the "Ernst Boehringer Institut für Arzneimittelforschung" of Bender&Co GmbH, Vienna / Austria.

Projects:

- Cloning, sequencing and bacterial expression of human and animal interferons (Interferon-α and interferon-ω).
- Bacterial expression of human Fce Receptor - soluble fragment
- Cloning, sequencing and bacterial expression of human vascular anticoagulant protein.
- Cloning and bacterial expression of the human TNF(Tumor Necrosis Factor)-binding protein.
- Development of GEMS (Gene expression modulation system) tester cell lines concerning the expression of cholesterol ester transfer protein (CETP) and apolipoprotein AI
- Oligonucleotide synthesis as a service for the individual molecular biology laboratories of the Ernst Boehringer Institut für Arzneimittelforschung (since 1985).
- DNA sequencing using fluorescence in combination with an automated sequencer as a service for individual molecular

biology laboratories of the Ernst Boehringer Institut für Arzneimittelforschung (since 1989).

- Molecular Biology part of TNF-alfa EG dossier (Sequencing, restriction mapping, copy number)
- EMAPII, an anti-angiogenic cytokine (expression in E.coli, genomic characterization, project coordination)

Since 1994 Group Leader in the department FEM

Since 1996 Project coordinator - Neoangiogenesis

R.Hauptmann, list of publications:

- R.Hauptmann, A.P.Czernilofsky, H.O.Voorma, G.Stöffler and E.Kuechler: Biochem.Biophys.Res.Comm. 56 (1974), 331-337: "Identification of a protein at the ribosomal donor-site by affinity labeling"

- E.Küchler, R.Hauptmann, A.P.Czernilofsky, I.Fiser, A.Barta, H.O.Voorma, G.Stöffler and K.H.Scheit: Acta Biol. Med.Germ. 33 (1974), 633-637: "A study of the structure of E.coli ribosomes by affinity labeling"

- R.Hauptmann, A.P.Czernilofsky, H.O.Voorma, G.Stöffler and E.Kuechler: Ribosomes and RNA Metabolism; Proceedings of the second international symposium on ribosomes and ribonucleic acid metabolism organized by the biological institute of the Slovak Academy of Science 2 (1975): 225-226: "Affinity labelling of the tRNA binding site of the E.coli ribosome"

- R.Hauptmann and E.Küchler: Veröffentlichungen der Universität Innsbruck 108 (1976), 57-59: "Untersuchungen des Peptidyltransferasezentrums des E.coli Ribosoms mittels Affinitätsmarkierung"

- R.Hauptmann, L.D.Clarke, R.C.Mountford, H.Bachmayer and J.W.Almond: J.Gen.Virol. 64 (1983), 215.220: "Nucleotide sequence of the Haemagglutinin gene of Influenza Virus A/England/321/77"

- A.J.Cann, G.Stanway, R.Hauptmann, P.D.Minor, G.C.Schild, L.D.Clarke, R.C.Mountford and J.W.Almond: Nucleic Acids Res. 11 (1983), 1267-1281: "Poliovirus type 3: molecular cloning of the genome and nucleotide sequence of the region encoding the protease and polymerase proteins

- P.D.Minor, G.C.Schild, J.Bootman, D.M.A.Evans, M.Ferguson, P.Reeve and M.Spitz, G.Stanway, A.J.Cann, R.Hauptmann, L.D.Clarke, R.C.Mountford and J.W.Almond: Nature 301 (1983), 674-679: "Location and primary structure of a major antigenic site for poliovirus neutralization"

- G.Stanway, A.J.Cann, R.Hauptmann, P.Hughes, L.D.Clarke, R.C.Mountford, P.D.Minor, G.C.Schild and J.W.Almond: Nucleic Acids Res. 11 (1983), 5629-5643: "The nucleotide sequence of polivirus type 3 leon 12 a_1b: comparison with poliovirus type 1"

- G.Stanway, A.J.Cann, R.Hauptmann, R.Mountford, L.D.Clarke, P.Reeve, P.D.Mino, G.C.Schild and J.W.Almond: Eur.J.Biochem. 135 (1983), 529-533: " Nucleic acid sequence

of the region of the genome encoding capsid protein VP1 of neurovirulent and attenuated type 3 polioviruses"

- J.W.Almond, A.J.Cann, P.D.Minor, P.Reeve, G.C.Schild, **R.Hauptmann** and G.Stanway Reviews of Infectious Diseases 6 Suppl.2 (1984), S487-S493: "Nucleotide sequence from neurovirulent and attenuated strains of type 3 poliovirus"

- **R.Hauptmann** and P.Swetly: Nucleic Acids Res. 13 (1985), 4739-4749: "A novel class of human type I interferons"

- **R.Hauptmann**, E.Ostermann, C.Pieler, W.Spevak and P.Swetly: The 1985 TNO-ISIR Meeting in the Interferon System (1985), 63: "A novel class of human type I interferons"

- **R.Hauptmann**: Wiener Klinische Wochenschrift 7/8 (1986), 158-160: "Escherichia coli in der Gentechnik"

- A.Himmler, **R.Hauptmann**, G.R.Adolf and P.Swetly: DNA 5 (1986), 345-356: "Molecular cloning and expression in Escherichia coli of equine type I interferons"

- **R.Hauptmann**, E.Ostermann, C.Pieler, W.Spevak and P.Swetly: Bundesministerium für Wissenschaft und Forschung: Informationsveranstaltung Biotechnologie und Gentechnik (1987): "Eine neue Klasse humaner Typ I Interferone"

- A.Himmler, **R.Hauptmann**, G.R.Adolf and P.Swetly: J.Interferon Res. 7 (1987), 173-183: "Structure and expression in Escherichia coli of canine interferon-α genes"

- I.Maurer-Fogy, C.P.M.Reutelingsperger, J.Pieters, G.Bodo, C.Stratowa and **R.Hauptmann**: Eur.J.Biochem. 174 (1988), 585-592: "Cloning and expression of cDNA for human vascular anticoagulant, a Ca^{2+}-dependent phospholipid-binding protein".

- **R.Hauptmann**, I.Maurer-Fogy, G.Bodo, C.Stratowa, J.Pieters and C.P.M.Reutelingsperger: Biol.Chem.Hoppe-Seyler 369 (1988),832: "Cloning and expression of the cDNA for human vascular anticoagulant, a Ca^{++}-dependent phospholipid binding protein"

- G.Bodo, **R.Hauptmann**, G.R.Adolf and I.Maurer-Fogy: Highlights of Modern Biochemistry (VSP International Science Publishers, Ed.: A.Kotyk, J.Skoda, V.Paces and V.Kostka) (1989), 1227-1236: "Human interferon-omega, a new component of leukocyte interferon"

- N.Uchibayashi, H.Kikutani, E.L.Barsumian, **R.Hauptmann**, F.J.Schneider, R.Schwendenwein, W.Sommergruber, W.Spevak, I.Maurer-Fogy, M.Suemura and T.Kishimoto: J.Immunology 142 (1989), 3901-3908: "Recombinant soluble Fce receptor II (FcsRII/CD23) has IgE binding activity but no B cell growth promoting activity"

- R.Hauptmann, I.Maurer-Fogy, E.Krystek, G.Bodo, H.Andree and C.P.M.Reutelingsperger: Eur.J.Biochem. 185 (1989) 63-71: "Vascular anticoagulant-β; a novel human Ca^{2+}/phospholipid binding protein that inhibits coagulation and phospholipase A2 activity"

- C.Pieler and R.Hauptmann: J.Interferon Res. 9, suppl.2 (1989), S181: "Study on the expression of human interferons α2 and ω1"

- C.P.M.Reutelingsperger, R.van Gool, J.Pieters, R.Hauptmann and H.C.H.Hemker: Thromb. Haemost. 62 (1) (1989), 385: "Inhibition of the procoagulant activity of the endotoxin stimulated endothelial cell by vascular anticoagulant (VAC)"

- C.P.M.Reutelingsperger, R.van Gool, R.Hauptmann and H.C.H.Hemker: Thromb. Haemost. 62 (1) (1989), 492: "Vascular anticoagulant: Its synthesis and its localisation in cultured human vascular endothelial cells"

- H.A.MAndree, C.P.M.Reutelingsperger, R.Hauptmann, H.C.Hemker, W.T.Hermens and G.M.Willems: J.Biol.Chem. 265 (1990), 4923-4928: "Binding of vascular anticoagulant α (VACα) to planar phospholipid bilayers"

- A.Himmler, I.Maurer-Fogy, M.Krönke, P.Scheurich, K.Pfizenmaier, M.Lantz, I.Olsson, R.Hauptmann, C.Stratowa and G.R.Adolf: DNA 9 (1990), 705-715: "Molecular cloning and expression of human rat tumor necrosis factor receptor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein"

- H.A.M.Andree, C.P.M.Reutelingsperger, R.Hauptmann, W.T.Hermens and G.M.Willems: Brit. J. Haematol. 76 Suppl.1 (1990), 13: "Annexin V (Vascular Anticoagulant alpha): Inhibition of the Prothrombinase Complex Activity"

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- C.P.M.Reutelingsperger, W.vanHeerdes, R.Hauptmann, C.Maassen, R.G.J.vanGool, P.deLeeuw and A.Tiebosch: FEBS Letters 348 (1994), 120-124: "Differential tissue expression of Annexin VIII in Human"

- S. Mörwald and R.Hauptmann: Atherosclerosis 109 (1994), 140 (Poster abstract):"Isolation and characterization of a human cytidine deaminase cDNA (apolipoprotein B mRNA editing enzyme)"

- C.-G. Liu, C. Maercker, M.J.Castañon, R.Hauptmann and G.Wiche: Proc. Natl. Acad. Sci. USA 93 (1996), 4278-4283: "Human plectin: Organisation of the gene, sequence analysis, and chromosomal localization (8q24)"

- S.Mörwald and R.Hauptmann: DNA and Cell Biol. (1996) submitted: "Two Different mRNA Variants for the Apolipoprotein B Editing Enzyme (apobec-1) in Human and Rat Gut"

B

Construction of expression vectors pDH10 (phoA promoter-STII-IFNα2c) and pDH11(trp promoter-STII-IFNα2c)

All cloning procedures were essentially performed following standard protocols ("Molecular cloning - a laboratory manual" Sambrook,J., Fritsch, E.F., & Maniatis T. (1989), Cold Spring Harbor Laboratory Press (1989).

pRH284/T: The promoter plasmid pRH284/T was generated in an analogous way as the promoter plasmid pRH281/5 (Case 12/069; DE-OS 38 10 474). A set of ligated oligonucleotides (phoA1-phoA10) was ligated between the EcoRI and ClaI sites of pAT153:

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:->phoA1
AATTGGAGATTATCGTCACTGCAATGCTTCGCAATATGGCGCAAAATGAC
    CCTCTAATAGCAGTGACGTTACGAAGCGTTATACCGCGTTTTACTG


          :->phoA3
CAACAGCGGTTGATTGATCAGGTAGAGGGGGCGCTGTACGAGGTAAAGCC
GTTGTCGCCAACTAACTAGTCCATCTCCCCCGCGACATGCTCCATTTCGG
          phoA2<-:

                        :->phoA5
CGATGCCAGCATTCCTGACGACGATACGGAGCTGCTGCGCGATTACGTAA
GCTACGGTCGTAAGGACTGCTGCTATGCCTCGACGACGCGCTAATGCATT
                        phoA4<-:

                                  :->phoA7
AGAAGTTATTGAAGCATCCTCGTCAGTAAAAAGTTAATCTTTTCAACAGC
TCTTCAATAACTTCGTAGGAGCAGTCATTTTTCAATTAGAAAAGTTGTCG
                                  phoA6<-:

                                            :->phoA9
TGTCATAAAGTTGTCACGGCCGAGACTTATAGTCGCTTTGTTTTTATTTT
ACAGTATTTCAACAGTGCCGGCTCTGAATATCAGCGAAACAAAAATAAAA
                                            phoA8<-:


TTAATGTATTTGCTCGAGAGGTTGAGGTGATTTTATGAGCTCGAATTCATC
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AATTACATAAACGAGCTCTCCAACTCCACTAAAATACTCGAGCTTAAGTAGCT
                                                phoA10<-:
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The resulting plasmid (pRH284) was then modified in the analogous way as pRH 281/5 by replacing the HindIII-SalI part of pRH284 with the oligonucleotide paie EBI-456/EBI-459, therby introducing the transcription terminator of phoA (H.Shuttleworth et al, Nucl.Acids Res. 14 (1986), 8689; C.N.Chang et al., Gene 44 (1986), 121-125).

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:->EBI-456
AGCTTGGATCCGTCGACCGCGCCCGGCAGTGAATTTTCGCTGCCGGGTGG
    ACCTAGGCAGCTGGCGCGGGCCGTCACTTAAAAGCGACGGCCCACC

TTTTTTTGCTGC
AAAAAAACGACGAGCT
      EBI-459<-:
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The resulting plasmid was named pRH 284/T.

STII-IFNα2c: The construction of the expression cassette containing the phosphatase promotor, the STII leader and the human IFN-a2c was performed by SOE-PCR (Splicing by overlap extension, Ho et al., 1989). The IFN-α2c sequence was PCR-amplified from the HindIII-linearized bacterial expression construct pER21/1 (Dworkin-Rastl, E., Swetly, P. & Dworkin, M.B. (1983) Gene 21, 237-248) using the 5' primer (ATGCCTATGCATGTGATCTGCCTCAAA-CCCACAGC) and the 3' primer (GACTTCAGAAGCTTCTGCAGTTA-CGATCGTTATCATTCCTTACTTCTTAAACTTTC, Hind III site underlined). The phosphatase promotor (Chang et al., 1986, Shuttleworth et al., 1986) and the STII leader (Lee, C.H., Mosely, S.L., Moon, H.W., Whipp, S.C., Gyles, C.L. & So, M. (1983) Infection & Immunity 42, 264-268; Picken, R.N., Mazaitis, A.J., Maas, W.K., Rey, M. & Heyneker, H. (1983) Infection & Immunity 42, 269-275) were amplified from the PvuI-linearized pCF2 (expression vector for human IFN-ω1 spliced to the STII leader sequence, total sequence found in file pCF2Seq.DOC) using the 5' (CGTCTTCAAGAATTCGAGATTATCG, EcoRI site underlined) and 3' (GGCAGATCACATGCATAGGCATTTGTAGCAATAG) primers. The purified PCR products were combined and amplified using the 3' primer of the first and the 5' primer of the second PCR reaction. The EcoRI/HindIII-cut

PCR product was cloned into the corresponding sites of Bluescribe M13$^+$; the nature of the insert was verified by sequencing (pBS-STII-IFNα2c).

pDH10, pDH11: The XhoI-HindIII fragment from pBS-STII-IFNα2c was isolated and ligated into XhoI-HindIII doubly restricted pRH284/T (phoA promoter construct, pDH10) or into XhoI-HindIII doubly restricted pRH281/5 (trp promoter construct, pDH11).

Both plasmids were used to transform E.coli HB101 .

Fermentation and Extraction

1. HB101/pDH10 (phoA-promoter)

Medium: Na^+, K^+, NH_4^+, Mg^{++}, Ca^{++}, $SO_4^=$, PO_4 in limiting concentration, Cl^-
vitamins, trace elements,
yeast extract, glucose,

Fermentation parameters: temperature 28°C, pH = 6,5
induction of IFN-α_2 expression takes place by phosphate depletion in the medium because of growth of E. coli

Extraction of biomass: a) 10 minutes in 1% SDS at 70°C in the water bath or
b) High pressure homogenisator at 1600 bar

Yield: 0,1 to 0,2 mg/l.OD or g/kg biomass, resp.

2. HB101/pDH11 (trp-promoter)

Medium: Na^+, K^+, NH_4^+, Mg^{++}, Ca^{++}, $SO_4^=$, PO_4, Cl^-
vitamins, trace elements,
yeast extract, glucose,

Fermentation parameters: Temperatur 28°C, pH = 6,5
Induction of IFN-α_2 expression with 3-β-indoleacrylic acid at start of fermentation;

Extraction of biomass: a) 10 minutes in 1% SDS at 70°C in the water bath or
b) High pressure homogenisator at 1600 bar

Yield: ca. 0,06 mg/l.OD or g/kg Biomasse, resp.

ELISA: biomass was diluted and measured in an ELISA against pure IFN-a2c as standard.